Unaltered prion disease in mice lacking developmental endothelial locus–1

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Abstract: Progression of prion diseases is driven by the accumulation of prions in the brain. Ablation of microglia or deletion of the eat-me-signal, milk-fat globule epidermal growth factor VIII (Mfge8), accelerates prion pathogenesis, suggesting that microglia defend the brain by phagocytosing prions. Similar to Mfge8, developmental endothelial locus–1 (Del-1) is a secreted protein that acts as an opsonin bridging phagocytes and apoptotic cells to facilitate phagocytosis. We therefore asked whether Del-1 might play a role in controlling prion pathogenesis. We assessed the anti-inflammatory and phagocytosis-promoting functions of Del-1 in prion disease and determined whether Del-1 complements Mfge8 in prion clearance in mice with a C57BL/6J genetic background. We found that Del-1 deficiency did not change prion disease progression or lesion patterns. In addition, prion clearance and scrapie prion protein deposition were unaltered in Del-1–deficient mice. In addition, prion-induced neuroinflammation was not affected by Del-1 deficiency. We conclude that Del-1 is not a major determinant of prion pathogenesis in this context.

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Negative results

Unaltered prion disease in mice lacking developmental endothelial locus−1

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ABSTRACT

Progression of prion diseases is driven by the accumulation of prions in the brain. Ablation of microglia or deletion of the eat-me-signal, milk-fat globule epidermal growth factor VIII (Mfge8), accelerates prion pathogenesis, suggesting that microglia defend the brain by phagocytosing prions. Similar to Mfge8, developmental endothelial locus−1 (Del-1) is a secreted protein that acts as an opsonin bridging phagocytes and apoptotic cells to facilitate phagocytosis. We therefore asked whether Del-1 might play a role in controlling prion pathogenesis. We assessed the anti-inflammatory and phagocytosis-promoting functions of Del-1 in prion disease and determined whether Del-1 complements Mfge8 in prion clearance in mice with a C57BL/6J genetic background. We found that Del-1 deficiency did not change prion disease progression or lesion patterns. In addition, prion clearance and scrapie prion protein deposition were unaltered in Del-1−deficient mice. In addition, prion-induced neuroinflammation was not affected by Del-1 deficiency. We conclude that Del-1 is not a major determinant of prion pathogenesis in this context.

1. Introduction

Prion diseases are lethal neurodegenerative disorders that affect both animals and humans (Aguzzi et al., 2013b). Prion diseases are characterized by deposition of the scrapie prion protein (PrP^{Sc}), a misfolded form of the cellular prion protein, in the central nervous system (CNS) (Aguzzi and Zhu, 2012). The accumulation of PrP^{Sc} within the CNS is accompanied by astrogliosis, microglial activation, and neuronal loss.

Microglia are the main innate immune cells in the CNS, exerting a defensive role against prions (Aguzzi and Zhu, 2017; Falsig et al., 2008; Zhu et al., 2016). However, the molecular mechanisms underlying prion clearance by microglia are not entirely clear. We have reported enhanced prion pathogenesis in mice lacking the astrocytes-borne opsonin protein, milk-fat globule epidermal growth factor (EGF) VIII (Mfge8). However, the deficiency of Mfge8 is deleterious only in C57BL/6J × 129Sv but not in C57BL/6J mice (Kranich et al., 2008, 2010), suggesting the existence of additional factors involved in prion clearance (Aguzzi et al., 2013a). Developmental endothelial locus−1 (Del-1) was originally found as an endothelial cell−derived extracellular matrix protein structurally containing 3 EGF-like repeats and 2 discoidin I-like domains. As a structural homolog of Mfge8, Del-1 can also bridge apoptotic cells and phagocytes to facilitate uptake and removal of apoptotic cells (Hanayama et al., 2004a). Del-1 has also appeared as an anti-inflammatory molecule (Choi et al., 2008, 2015).

In this study, we aimed to investigate whether the phagocytosis-promoting and anti-inflammatory functions of Del-1 may have an impact on prion pathogenesis and to determine whether Del-1 complements Mfge8 in prion clearance in mice with a C57BL/6J genetic background. We observed that Del-$1^{-/-}$ mice displayed disease progression and lesion patterns similar to those of their heterozygous (Del-$1^{+/-}$) and wild-type (Del-$1^{+/+}$) littermates. Importantly, PrP^{Sc} deposition was not altered by Del-1 deficiency, suggesting that Del-1 is not involved in prion clearance in vivo. Furthermore, Del-1 deficiency did not overtly affect prion-induced neuroinflammation. On the basis of these observations, we conclude with a high degree of confidence that Del-1 is not a major determinant of prion clearance and does not impact prion pathogenesis in any measurable way. Del-1 does not complement Mfge8 in prion clearance in mice with a C57BL/6J genetic background.

2. Material and methods

2.1. Animals and ethical statement

Del-$1^{-/-}$ mice with a C57BL/6J genetic background (Choi et al., 2008) were backcrossed to C57BL/6J mice to obtain Del-$1^{+/-}$

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offspring, which were then intercrossed to generate Del-1$^{+/+}$ (wild type), Del-1$^{+/-}$ and Del-1$^{-/-}$ mice for experiments described here. Mfge8$^{-/-}$/Del-1$^{-/-}$ double knockout mice were generated by breeding Del-1$^{-/-}$ mice to Mfge8$^{-/-}$ mice (Hanayama et al., 2004b; Kranich et al., 2010).

All animal experiments were carried out in strict accordance with the rules and regulations for the Protection of Animal Rights (Tierschutzgesetz and Tierschutzverordnung) of the Swiss Bundesamt für Lebensmittelsicherheit und Veterinärwesen and were preemptively approved by the Animal Welfare Committee of the Canton of Zürich (permit # 41/2012).

2.2. Prion inoculation

Mice at the age of 6–8 weeks were intracerebrally (i.c.) inoculated with 30 μL of brain homogenate diluted in phosphate buffered saline with 5% bovine serum albumin and containing 3×10^5 LD50 units of the Rocky Mountain Laboratories scrapie strain (passage 6, thus called RML6). Mice were monitored and actions were taken to minimize animal suffering and distress according to details described previously (Zhu et al., 2015b).

2.3. Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the SYBR Green PCR Master Mix (Roche) on a ViiA7 Real-Time PCR system (Applied Biosystems) as described previously (Zhu et al., 2015a). Expression levels were normalized using glyceraldehyde-3-phosphate dehydrogenase.

2.4. Immunohistochemistry

Immunohistochemical staining was performed as described previously (Zhu et al., 2015a). Anti-PrP SAF-84 (1:200, SPI Bio, A03208), anti-GFAP antibody (1:300; DAKO, Carpinteria, CA, USA), and anti-Iba1 antibody (1:1000; Wako Chemicals GmbH, Germany) were used to stain PrP^{Sc}, astrocytes, and microglia, respectively.

2.5. Western blot analysis

Proteins were loaded and separated on a 12% Bis-Tris polyacrylamide gel (NuPAGE, Invitrogen) and processed as described previously (Zhu et al., 2015a). Primary antibodies anti-PrP antibody POM1 (400 ng mL^{-1}), anti-NeuN clone EPR12763 (1:3000, Abcam, ab177487), anti-synaptophysin clone 2/synaptophysin (1:10,000, BD Biosciences, 611880), anti-synapsin I (1:2000, Millipore, AG, USA, AB1543), anti-Iba1 antibody (1:1000; Wako Chemicals GmbH, Germany, 019–19,741), anti-GFAP antibody (D1F4Q) XP Rabbit mAb (1:3000; Cell Signaling Technology, 12,389), anti-Mfge8 antibody (1:1000; R&D Systems, AF2805), anti-actin antibody (1:10,000, Millipore), and secondary antibody horseradish peroxidase–conjugated goat anti-mouse IgG (1:10,000, Jackson ImmunoResearch, 115-035-003), goat anti-rabbit IgG (1:10,000, Jackson ImmunoResearch, 111-035-045), and donkey anti-goat IgG(1:10,000, Jackson ImmunoResearch, 705-035-147) were used. For proteinase K (PK) digestion, samples were adjusted to 20 μg protein in 20 μL and digested with 25 μg mL^{-1} proteinase K for 30 minutes at 37 °C.

2.6. Real-time quaking-induced conversion assay

Real-time quaking-induced conversion assay (RT-QuIC) assays of prion-infected mouse brain homogenates were performed as previously described (Frontzek et al., 2016).

3. Results

3.1. Del-1 deficiency does not affect prion progression and lesion pattern

Del-1 is highly expressed in mouse brains and mainly expressed by neurons (Supplemental Figs. 1–5). Del-1 deficiency did not change the transcription of *Prnp* and protein levels of the cellular prion protein in brains (Fig. 1A and B), which are major determinants of susceptibility to prion diseases. To determine the role of Del-1 in prion diseases, we i.c. inoculated RML6 prions (a prion strain derived from the Rocky Mountain Laboratory, passage 6) into Del-1$^{+/+}$, Del-1$^{+/-}$, and Del-1$^{-/-}$ littermates. We observed that Del-1$^{+/+}$, Del-1$^{+/-}$, and Del-1$^{-/-}$ mice succumbed to prion disease at a similar rate [median survival: 186 dpi for female Del-1$^{+/+}$ mice (n = 6), 191.5 dpi for female Del-1$^{+/-}$(n = 16), and 193.5 dpi for female Del-1$^{-/-}$ mice (n = 8), $p = 0.26$; median survival 196.5 dpi for male Del-1$^{+/+}$ mice(n = 14), 195 dpi for male Del-1$^{+/-}$(n = 19), and 197 dpi for male Del-1$^{-/-}$ mice (n = 11), $p = 0.87$] (Fig. 1C). These results indicate that Del-1 deficiency does not significantly affect prion progression.

To determine whether Del-1 and Mfge8 work redundantly in prion clearance, we bred Del-1$^{-/-}$ mice and Mfge8$^{-/-}$ mice to obtain Mfge8$^{-/-}$/Del-1$^{-/-}$ double knockout mice with a C57BL/6J genetic background and i.c. inoculated RML6 prions. Again, we observed a similar prion progression in Mfge8$^{-/-}$/Del-1$^{-/-}$ double knockout and wild-type (Mfge8$^{+/+}$/Del-1$^{+/+}$) littermates (median survival: 182.5 dpi for female Mfge8$^{+/+}$/Del-1$^{+/+}$ mice [n = 4], 181.5 dpi for female Mfge8$^{-/-}$/Del-1$^{-/-}$ mice [n = 4], $p = 0.72$) (Supplemental Fig. 6), suggesting that neither Del-1 nor Mfge8 is an important determinant of prion pathogenesis in mice within the C57BL/6J genetic background.

We then analyzed the histology of brain sections prepared from prion-infected terminally sick Del-1$^{+/+}$, Del-1$^{+/-}$, and Del-1$^{-/-}$ mice. The typical histological features of prion disease, including spongiform vacuolation, were observed in all mice with different genotypes (Fig. 1D). Lesion pattern analysis failed to find any qualitative differences between the 3 groups (Fig. 1D). These results indicate that Del-1 deficiency does not affect prion-induced lesion pattern in mouse brains. We then performed Western blots for neuronal markers (NeuN, synaptophysin, and synapsin I) to assess whether Del-1 deficiency affects prion-induced neuronal death; we failed to observe obvious differences between Del-1$^{+/+}$, Del-1$^{+/-}$, and Del-1$^{-/-}$ mouse brains (Fig. 1E, full images of the cropped Western blots are shown in Supplementary Fig. 7). Therefore, Del-1 deficiency does not alter prion-induced neuronal death.

3.2. Del-1 deficiency does not alter PrPSc accumulation

We next sought to determine whether Del-1 is involved in prion clearance in mouse brains. We performed PrP^{Sc} staining on brain sections prepared from prion-infected terminally sick Del-1$^{+/+}$, Del-1$^{+/-}$, and Del-1$^{-/-}$ mice. However, we observed similar PrP^{Sc} deposition level in all 3 groups (Fig. 2A). We next carried out Western blot to detect proteinase K–resistant PrP^{Sc} in brains of prion-infected terminally sick Del-1$^{+/+}$, Del-1$^{+/-}$, and Del-1$^{-/-}$ mice. Again, we found similar levels of PrP^{Sc} in mouse brains of the 3 genotypes (Fig. 2B). Furthermore, we assessed the seeding activity in brain homogenates of prion-infected terminally sick Del-1$^{+/+}$, Del-1$^{+/-}$, and Del-1$^{-/-}$ mice by RT-QuIC. We detected a similar prion 50% seeding dose in all 3 groups (Fig. 2C). These results suggest that Del-1 does not contribute to prion clearance and PrP^{Sc} accumulation in mouse brains.

To test the possibility that Del-1 deficiency could upregulate Mfge8 expression through a compensatory mechanism, thereby

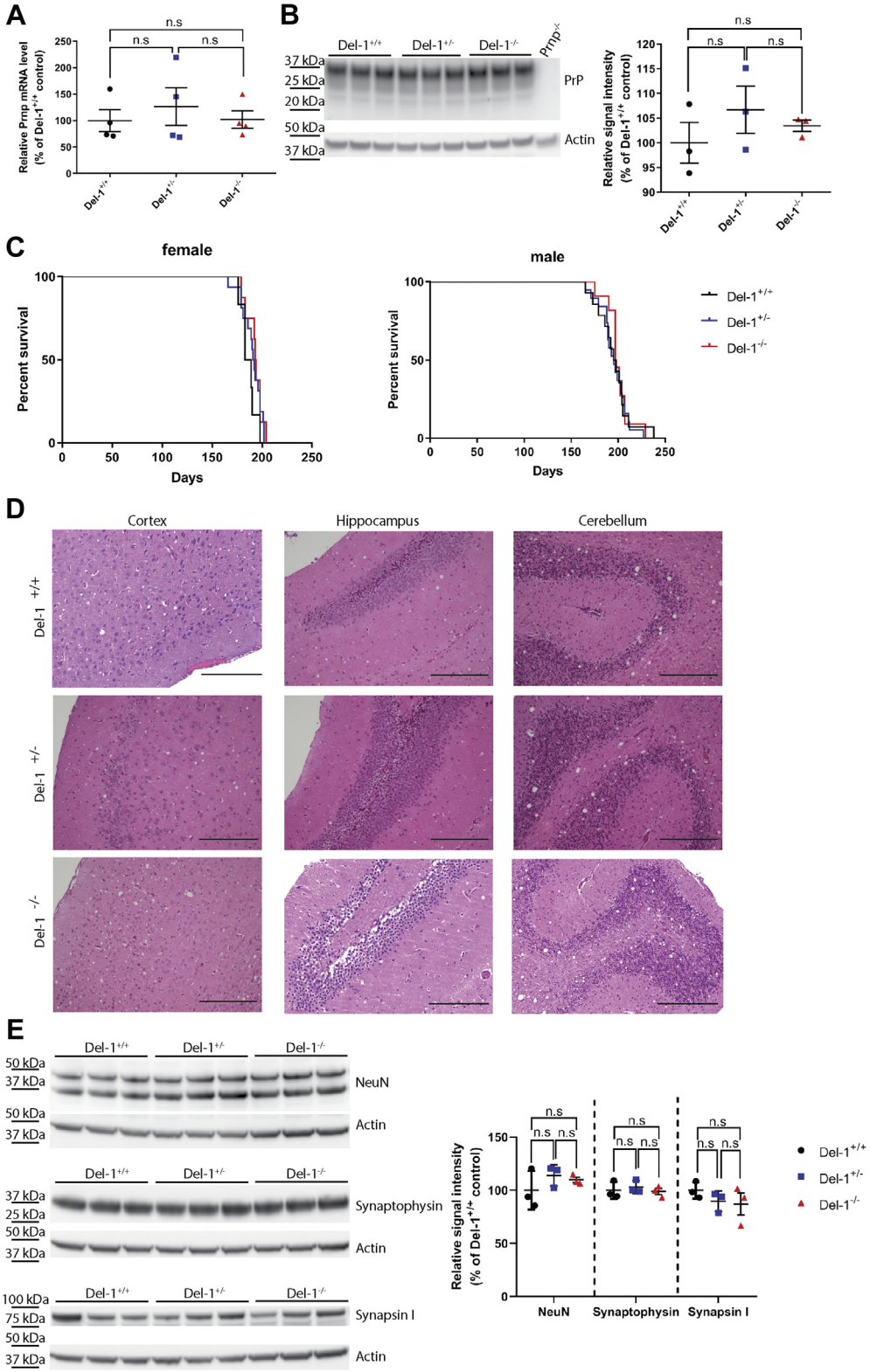

Fig. 1. (A and B) *Prnp* qRT-PCR (A) and PrP^C Western blot (B left; densitometric quantification of the Western blot in B right) in Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. No significant difference in *Prnp* mRNA and PrP^C protein was observed between Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. Relative expression was represented as percentage of average values in Del-$1^{+/+}$ control mice. (C) Survival curve of Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ littermates intracerebrally inoculated with RML6. There was no significant difference between 3 groups in both genders (n = 6–19, n.s $p > 0.05$). (D) Representative histology of terminally sick mouse brains from Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ littermates stained with hematoxylin and eosin (H&E). There was no obvious difference between the 3 groups with respect to vacuolation or lesion pattern in the cortex, hippocampus, and cerebellum, scale bars: 200 μm. (E) Left: Western blot for NeuN, synaptophysin, and synapsin I in prion-infected Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. Right: densitometric quantification of the NeuN, synaptophysin, and synapsin I Western blots. n = 3 for each genotype. The relative signal intensity was represented as percentage of average values in Del-$1^{+/+}$ control mice. There was no significant difference between 3 groups in both genders (n = 3, n.s $p > 0.05$). Abbreviations: Del-1, developmental endothelial locus–1; PrP^C, cellular prion protein; qRT-PCR, quantitative real-time polymerase chain reaction; RML6, Rocky Mountain Laboratories scrapie strain, passage 6; WT, wild type.

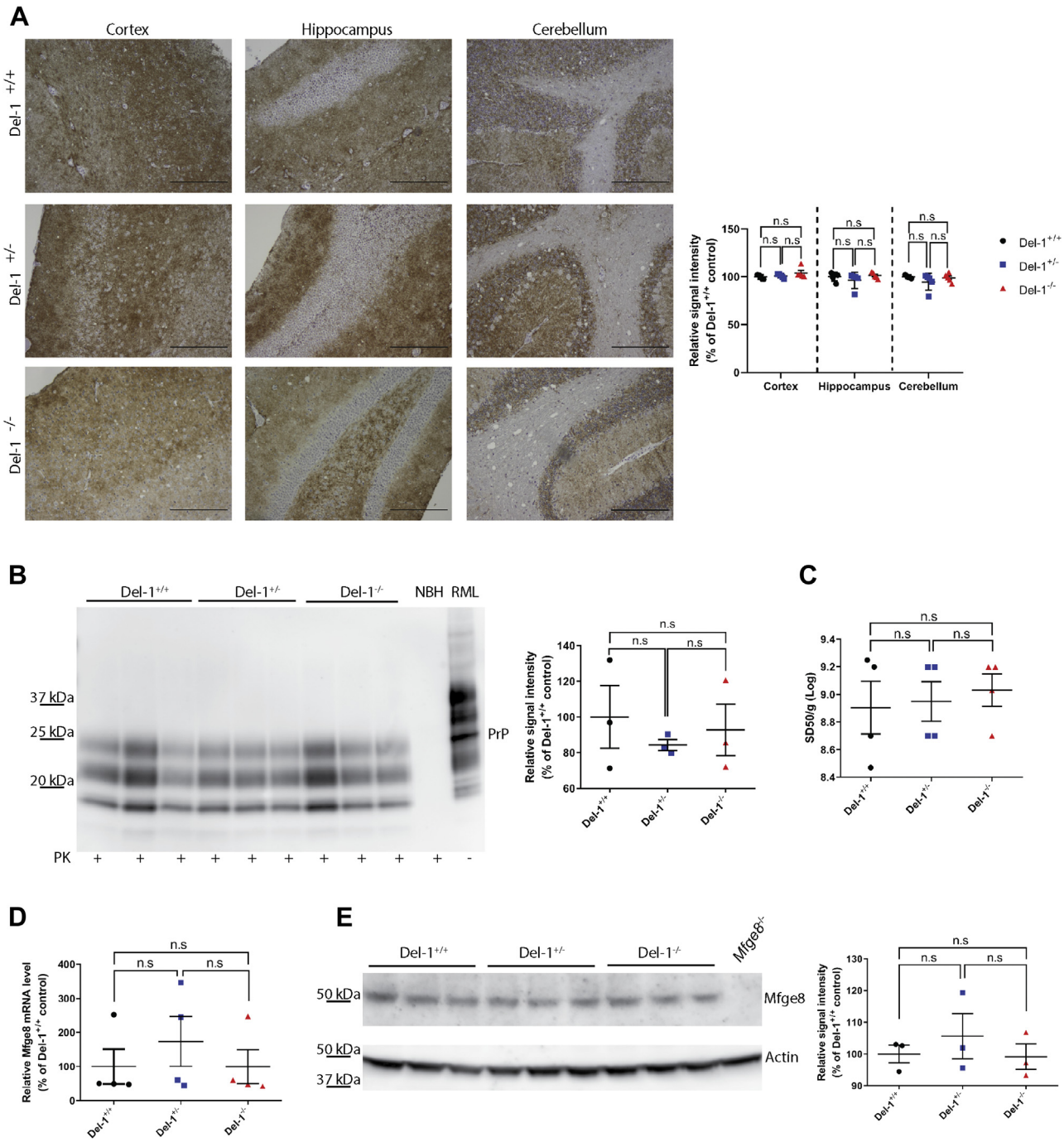

Fig. 2. (A) Left: Representative histology of terminally sick mouse brains from Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ littermates stained for SAF84. There was no obvious difference between the 3 groups in PrP^{Sc} deposition in the cortex, hippocampus, and cerebellum, scale bars: 200 μm. Right: quantification of the SAF84 staining in the cortex, cerebellum, and hippocampus of Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ littermates (n = 5, n.s $p > 0.05$). Relative signal intensity was represented as percentage of average values in Del-$1^{+/+}$ control mice. (B) Left: Western blot for proteinase K–resistant PrP^{Sc} in terminally sick mouse brains. Right: densitometric quantification of the Western blot showed no significant difference between Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ littermates (n = 3, n.s $p > 0.05$). (C) RT-QuIC assay of terminally sick mouse brains from Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ littermates showed similar level of 50% prion seeding dose in these 3 groups (n = 4, n.s $p > 0.05$). (D and E) *Mfge8* qRT-PCR (D) and Mfge8 Western blot (E left; densitometric quantification of the Western blot in E right) in Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. No significant difference of *Mfge8* mRNA and Mfge8 protein was observed between Del-$1^{+/+}$ (WT), Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. Abbreviations: Del-1, developmental endothelial locus–1; NBH, noninfectious brain homogenates; Mfge8, milk-fat globule EGF factor VIII; qRT-PCR, quantitative real-time polymerase chain reaction; RT-QuIC, real-time quaking-induced conversion assay; WT, wild type.

blunting the acceleration of prion progression in Del-$1^{-/-}$ mice, we assessed the expression of Mfge8 in Del-$1^{+/+}$, Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. Both qRT-PCR and Western blot failed to detect obvious changes in Mfge8 mRNA and protein levels in Del-$1^{-/-}$ mouse brains (Fig. 2D and E, full image of the cropped Western blots is shown in Supplementary Fig. 7), suggesting that the unchanged prion pathogenesis in Del-$1^{-/-}$ mice is not due to a compensatory effect of its homolog Mfge8.

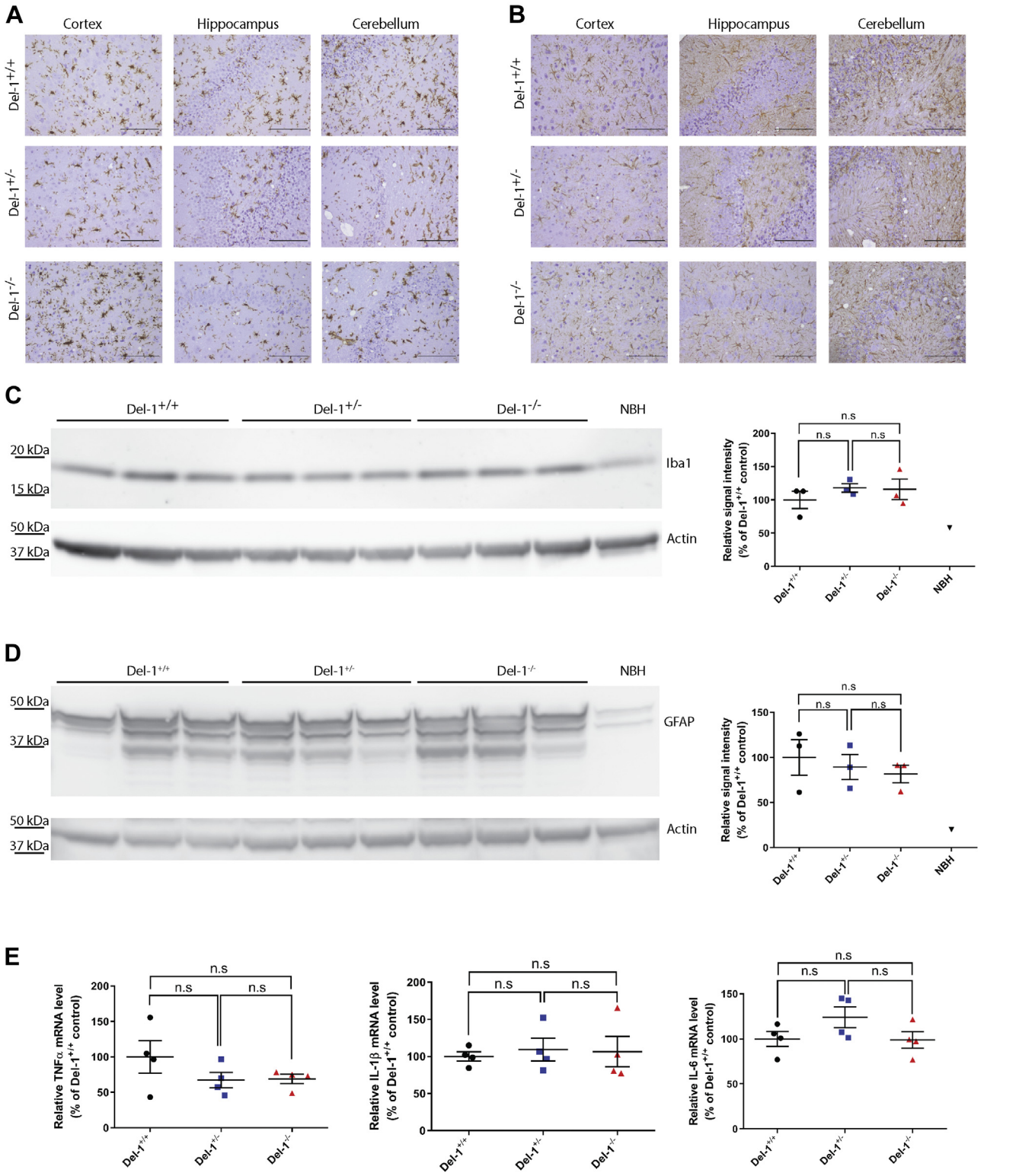

Fig. 3. (A and B) Representative immunohistochemical staining for Iba1 (A) and GFAP (B) in the cortex, hippocampus, and cerebellum of terminally sick mouse brains from Del-1$^{+/+}$ (WT), Del-1$^{+/-}$, and Del-1$^{-/-}$ littermates, scale bars: 100 μm. (C) Left: Western blot for Iba1 in terminally sick mouse brains. Right: densitometric quantification of the Western blot revealed no significant difference in Iba1 levels between Del-1$^{+/+}$ (WT), Del-1$^{+/-}$, and Del-1$^{-/-}$ littermates (n = 3, n.s $p > 0.05$). (D) Left: Western blot for GFAP in terminally sick mouse brains. Right: densitometric quantification of the Western blot showed no significant difference of GFAP levels between Del-1$^{+/+}$ (WT), Del-1$^{+/-}$, and Del-1$^{-/-}$ littermates (n = 3, n.s $p > 0.05$). (E) qRT-PCR of cytokines tumor necrosis factor α, interleukin 6, and interleukin-1β expression revealed similar expression levels of these cytokines in terminally sick Del-1$^{+/+}$ (WT), Del-1$^{+/-}$, and Del-1$^{-/-}$ littermates (n = 4, n.s $p > 0.05$). Abbreviations: Del-1, developmental endothelial locus−1; GFAP, glial fibrillary acidic protein; qRT-PCR, quantitative real-time polymerase chain reaction; WT, wild type.

3.3. Del-1 deficiency does not affect prion-induced neuroinflammation

To determine whether Del-1 also plays an anti-inflammatory effect in prion pathogenesis, we analyzed microglial activation and astrogliosis in prion-infected terminally sick Del-$1^{+/+}$, Del-$1^{+/-}$, and Del-$1^{-/-}$ mice. Histology failed to reveal overt differences in Iba1 (microglial marker) and glial fibrillary acidic protein (GFAP, astrocytic marker) immunoreactivity between Del-$1^{+/+}$, Del-$1^{+/-}$, and Del-$1^{-/-}$ mice (Fig. 3A and B). We then performed Western blots to detect Iba1 and GFAP protein in prion-infected terminally sick Del-$1^{+/+}$, Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. Again, we observed similar levels of Iba1 and GFAP in all 3 groups (Fig. 3C and D, full images of the cropped Western blots are shown in Supplementary Fig. 7). These results suggest that, in contrast to other models of neurological disease such as EAE, Del-1 deficiency does not overtly affect prion-induced microglial activation and astrogliosis.

We also performed cytokine profiling using qRT-PCR to assess the expression of proinflammatory cytokines including tumor necrosis factor α, interleukin-1β, and interleukin 6 in prion-infected terminally sick Del-$1^{+/+}$, Del-$1^{+/-}$, and Del-$1^{-/-}$ mouse brains. This experiment did not detect obvious differences in cytokine expression between the 3 groups (Fig. 3E), suggesting that Del-1 deficiency fails to alter prion-induced cytokine expression.

4. Discussion

Neuroinflammation, characterized by astrogliosis and microglial activation, is considered a hallmark of various neurodegenerative conditions including Alzheimer's disease and prion diseases (Aguzzi et al., 2013a). The molecular mechanisms by which microglia contribute to the pathogenesis of neurodegenerative conditions are subjected to extensive investigations. In this study, we aimed to study whether Del-1 plays phagocytosis-promoting and/or anti-inflammation functions in prion diseases. In particular, we sought to determine whether Del-1 complements Mfge8 in prion clearance in mice with a C57BL/6J genetic background, in which Mfge8 deficiency does not influence prion pathogenesis. We observed that Del-$1^{-/-}$ mice experienced a similar incubation time compared to Del-$1^{+/-}$ and wild-type littermates, suggesting that Del-1 deficiency does not affect prion disease progression. More importantly, the PrP^{Sc} level and prion seeding activity were not detectably affected by Del-1 deficiency, indicating that Del-1 is not a major contributor to prion clearance. Moreover, prion-induced astrocytosis and microglial activation were not altered in Del-$1^{-/-}$ mouse brains, suggesting that Del-1 deficiency does not overtly affect prion-induced neuroinflammation. Therefore, our study revealed that Del-1 is not a major determinant of prion pathogenesis and does not complement Mfge8 in prion clearance in mice with the C57BL/6J genetic background. Further studies are required to identify molecular mechanisms underlying microglial uptake and clearance of prions.

Disclosure

The authors have no actual or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.01.003.

References

Aguzzi, A., Barres, B.A., Bennett, M.L., 2013a. Microglia: scapegoat, saboteur, or something else? Science 339, 156–161.
Aguzzi, A., Nuvolone, M., Zhu, C., 2013b. The immunobiology of prion diseases. Nat. Rev. Immunol. 13, 888–902.
Aguzzi, A., Zhu, C., 2012. Five questions on prion diseases. PLoS Pathog. 8, e1002651.
Aguzzi, A., Zhu, C., 2017. Microglia in prion diseases. J. Clin. Invest. 127, 3230–3239.
Choi, E.Y., Chavakis, E., Czabanka, M.A., Langer, H.F., Fraemohs, L., Economopoulou, M., Kundu, R.K., Orlandi, A., Zheng, Y.Y., Prieto, D.A., Ballantyne, C.M., Constant, S.L., Aird, W.C., Papayannopoulou, T., Gahmberg, C.G., Udey, M.C., Vajkoczy, P., Quertermous, T., Dimmeler, S., Weber, C., Chavakis, T., 2008. Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. Science 322, 1101–1104.
Choi, E.Y., Lim, J.H., Neuwirth, A., Economopoulou, M., Chatzigeorgiou, A., Chung, K.J., Bittner, S., Lee, S.H., Langer, H., Samus, M., Kim, H., Cho, G.S., Ziemssen, T., Bdeir, K., Chavakis, E., Koh, J.Y., Boon, L., Hosur, K., Bornstein, S.R., Meuth, S.G., Hajishengallis, G., Chavakis, T., 2015. Developmental endothelial locus-1 is a homeostatic factor in the central nervous system limiting neuroinflammation and demyelination. Mol. Psychiatry 20, 880–888.
Falsig, J., Julius, C., Margalith, I., Schwarz, P., Heppner, F.L., Aguzzi, A., 2008. A versatile prion replication assay in organotypic brain slices. Nat. Neurosci. 11, 109–117.
Frontzek, K., Pfammatter, M., Sorce, S., Senatore, A., Schwarz, P., Moos, R., Frauenknecht, K., Hornemann, S., Aguzzi, A., 2016. Neurotoxic antibodies against the prion protein do not trigger prion replication. PLoS One 11, e0163601.
Hanayama, R., Tanaka, M., Miwa, K., Nagata, S., 2004a. Expression of developmental endothelial locus-1 in a subset of macrophages for engulfment of apoptotic cells. J. Immunol. 172, 3876–3882.
Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K., Koike, M., Uchiyama, Y., Nagata, S., 2004b. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science 304, 1147–1150.
Kranich, J., Krautler, N.J., Falsig, J., Ballmer, B., Li, S., Hutter, G., Schwarz, P., Moos, R., Julius, C., Miele, G., Aguzzi, A., 2010. Engulfment of cerebral apoptotic bodies controls the course of prion disease in a mouse strain-dependent manner. J. Exp. Med. 207, 2271–2281.
Kranich, J., Krautler, N.J., Heinen, E., Polymenidou, M., Bridel, C., Schildknecht, A., Huber, C., Kosco-Vilbois, M.H., Zinkernagel, R., Miele, G., Aguzzi, A., 2008. Follicular dendritic cells control engulfment of apoptotic bodies by secreting Mfge8. J. Exp. Med. 205, 1293–1302.
Zhu, C., Herrmann, U.S., Falsig, J., Abakumova, I., Nuvolone, M., Schwarz, P., Frauenknecht, K., Rushing, E.J., Aguzzi, A., 2016. A neuroprotective role for microglia in prion diseases. J. Exp. Med. 213, 1047–1059.
Zhu, C., Herrmann, U.S., Li, B., Abakumova, I., Moos, R., Schwarz, P., Rushing, E.J., Colonna, M., Aguzzi, A., 2015a. Triggering receptor expressed on myeloid cells-2 is involved in prion-induced microglial activation but does not contribute to prion pathogenesis in mouse brains. Neurobiol. Aging 36, 1994–2003.
Zhu, C., Schwarz, P., Abakumova, I., Aguzzi, A., 2015b. Unaltered prion pathogenesis in a mouse model of high-fat diet-induced insulin resistance. PLoS One 10, e0144983.